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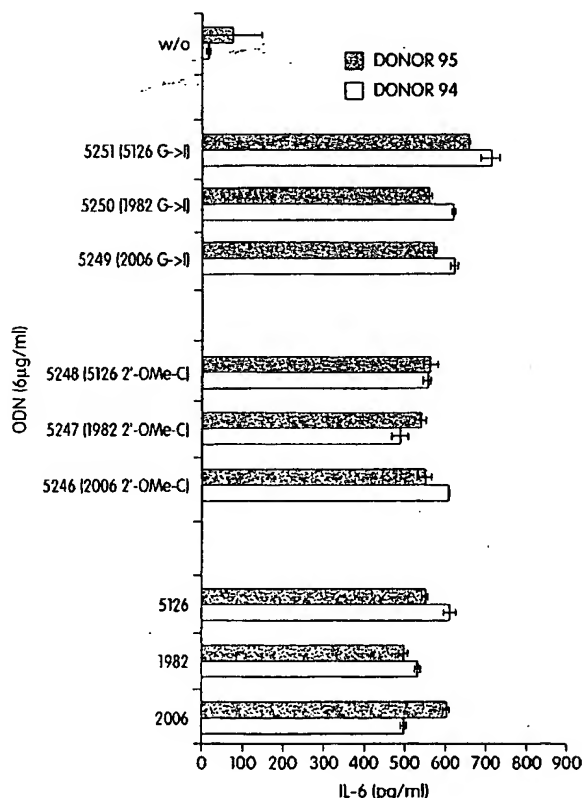
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60/254,341 8 December 2000 (08.12.2000) US
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- (74) Agents: JUMP, Timothy et al.; Venner, Shipley & Co., 20 Little Britain, London EC1A 7DH (GB).
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[Continued on next page]

(54) Title: CPG-LIKE NUCLEIC ACIDS AND METHODS OF USE THEREOF



(57) Abstract: Immunostimulatory compositions described as CpG-like nucleic acids are provided, including nucleic acids having immunostimulatory characteristics of CpG nucleic acid, despite certain substitutions of C, G, or C and G of the CpG dinucleotide. The substitutions can include, among others, exchange of methylated C for C, inosine for G, and ZpY for CpG, where Z is Cytosine or dSpacer and Y is inosine, 2-aminopurine, nebularine, or dSpacer. Also provided are methods for inducing an immune response in a subject using the CpG-like nucleic acids. The methods are useful in the treatment of a subject that has or is at risk of developing an infectious disease, allergy, asthma, cancer, anemia, thrombocytopenia, or neutropenia.

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## INTERNATIONAL SEARCH REPORT

 International Application No  
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 A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 7 C12N15/11 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GOECKERITZ B.E. ET AL.: "Multivalent cross-linking of membrane Ig sensitizes murine B cells to a broader spectrum of CpG-containing oligodeoxynucleotide motifs, including their methylated counterparts, for stimulation of proliferation and Ig secretion" INTERNATIONAL IMMUNOLOGY, vol. 11, no. 10, 1999, pages 1693-1700, XP000938323 the whole document --- -/--	1,2, 13-19, 21,38, 43-49

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

17 January 2003

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## INTERNATIONAL SEARCH REPORT

Interr I Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOGGS R.T. ET AL.: "Characterization and modulation of immune stimulation by modified oligonucleotides" ANTISENSE & NUCLEIC ACID DRUG DEVELOPMENT, vol. 7, 1 October 1997 (1997-10-01), pages 461-471, XP002053418 ISSN: 1087-2906 page 465, left-hand column page 467, left-hand column, paragraph 3 -right-hand column ---	1,13-15, 18,19, 37,38, 43,44, 48,72
A	YAMAMOTO T. ET AL.: "Lipofection of synthetic oligodeoxyribonucleotide having a palindromic sequence of AAGCTT to murine splenocytes enhances Interferon production and natural killer activity" MICROBIOLOGY AND IMMUNOLOGY, vol. 38, no. 10, 1994, pages 831-836, XP008012406 page 831 ---	
X	FELTQUATE D.M. AND ROBINSON H.L.: "Effect of CpG methylation on isotype and magnitude of antibody responses to influenza hemagglutinin-expressing plasmid" DNA AND CELL BIOLOGY, vol. 18, no. 9, 1999, pages 663-670, XP002226660 page 668, left-hand column; table 1 page 669, left-hand column ---	1,2,13, 18,19, 21,24, 38,43, 48-50, 52,75,76
E	WO 01 93905 A (CISTEM BIOTECHNOLOGIES GMBH (AT); SCHMIDT; LINGNAU; SCHELLACK; EGYED) 13 December 2001 (2001-12-13)  page 7, paragraph 2 page 34 -page 37; claims ---	1,13-18, 21,38, 43-46, 48-50, 52,75,76
A	KRIEG A.M. ET AL.: "Mechanisms and therapeutic applications of immune stimulatory CpG DNA" PHARMACOLOGY & THERAPEUTICS, vol. 84, 1999, pages 113-120, XP002226661 ---	
A	POLANCZYK M.: "Immunostimulatory effects of DNA and CpG motifs" CENTRAL EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 25, no. 3, 2000, pages 160-166, XP008012376 ---	
A	WO 99 62923 A (DYNAVAX TECHNOLOGIES CORPORATION (US); SCHWARTZ DAVID) 9 December 1999 (1999-12-09) -----	

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IB 01/02888

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 38 and 43-77 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
  
1-2, 38 (complete); 13-37, 43-77 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-2, 38 all totally; 13-37, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula:  
5' X1X2CGX3X4 3' wherein C is methylated and wherein X1-4 are nucleotides. Related compositions and methods thereof.

2. Claims: 3-5 all totally; 13-37 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula:  
5' X1X2CGX3X4 3' wherein C is 2'alkoxy cytosine and wherein X1-4 are nucleotides. Related compositions thereof.

3. Claims: 6-9, 13-37, 39, 40, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula:  
5' X1X2ZYZX3X4 3' wherein Z is cytosine and Y is 2-aminopurine and wherein X1-4 are nucleotides. Related compositions and methods thereof.

4. Claims: 6-9, 13-37, 39, 40, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula:  
5' X1X2ZYZX3X4 3' wherein Z is cytosine and Y is xanthosine or N7-methyl-xanthosine and wherein X1-4 are nucleotides. Related compositions and methods thereof.

5. Claims: 6-9, 13-37, 39, 40, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula:  
5' X1X2ZYZX3X4 3' wherein Z is cytosine and Y is nebularine and wherein X1-4 are nucleotides. Related compositions and methods thereof.

6. Claims: 6-9, 13-37, 39, 40, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula:  
5' X1X2ZYZX3X4 3' wherein Z is cytosine and Y is dSpacer and wherein X1-4 are nucleotides. Related compositions and methods thereof.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

7. Claims: 6, 8, 9, 13-37, 39, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula: 5' X1X2ZYX3X4 3' wherein Z is 2'-deoxyuridine (dU) or 5-fluoro-2'-dU and Y is inosine and wherein X1-4 are nucleotides. Related compositions and methods thereof.

8. Claims: 6, 8, 9, 13-37, 39, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula: 5' X1X2ZYX3X4 3' wherein Z is 2'-deoxyuridine (dU) or 5-fluoro-2'-dU and Y is 2-aminopurine and wherein X1-4 are nucleotides. Related compositions and methods thereof.

9. Claims: 6, 8, 9, 13-37, 39, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula: 5' X1X2ZYX3X4 3' wherein Z is 2'-deoxyuridine (dU) or 5-fluoro-2'-dU and Y is xanthosine or N7-methyl-xanthosine and wherein X1-4 are nucleotides. Related compositions and methods thereof.

10. Claims: 6, 8, 9, 13-37, 39, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula: 5' X1X2ZYX3X4 3' wherein Z is 2'-deoxyuridine (dU) or 5-fluoro-2'-dU and Y is nebularine and wherein X1-4 are nucleotides. Related compositions and methods thereof.

11. Claims: 6, 8, 9, 13-37, 39, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula: 5' X1X2ZYX3X4 3' wherein Z is 2'-deoxyuridine (dU) or 5-fluoro-2'-dU and Y is dSpacer and wherein X1-4 are nucleotides. Related compositions and methods thereof.

12. Claims: 10-12, 41, 42 all totally; 13-37, 43-77 all partially

A composition comprising an immunostimulatory nucleic acid having a sequence including at least the following formula: 5' X1X2CIX3X4 3' wherein C is cytosine and I is inosine and wherein X1-4 are nucleotides. Related compositions and methods thereof.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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